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RESPIRATION OF STORED WHEAT[1]

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INTRODUCTION

The preservation in storage of large quantities of thrashed grain involves certain difficulties. In addition to protection from vermin, it has long been known that the grain must be dry when stored, and the ingress of moisture prevented. The ancients were aware that damp grain, or similar vegetable material, will heat and become decomposed when stored in quantities. It is evident that in prehistoric times carefully constructed receptacles were employed for the protection of the reserve supplies of cereals. Those ancient people of India, the Hindus, sometimes resort to the use of receptacles which are submerged in cool water, reducing the temperature of the stored grain and also the supply of air. In modern practice carefully constructed tanks or silos, frequently of large capacity, are chiefly used for the storage of grain.

CAUSE OF THE HEATING OF GRAIN

The cause of heating of damp vegetable matter was not known, however, until comparatively recent times, and it is only within the last decade or two that any data have been accumulated which indicate the exact effect of various factors on the rate of heating of grain and similar material. That the phenomenon known as respiration is responsible for the heat energy released in a mass of damp grain is shown by modern research. Loew (*1899*)[2], in discussing the fermentation and heating of leaf tobacco, maintained that the release of energy and rise in temperature was occasioned by the activity of the oxidizing enzyms of the leaf cells. The microbial flora were not believed to play any considerable part in these changes. In advancing this view he opposed vigorously the bacterial-fermentation theory of Suchsland (*1891*).

Rahn (*1910*) states that the curve of the process of spontaneous heating of organic matter, including grain, would not in itself indicate whether

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[2] Bibliographic citations in parentheses (italic) refer to "Literature cited," pp. 710-713.

the heat was produced by chemical or microbial causes. Gore (*1911, p. 33*) concluded that in self-heating, physiological processes are probably the first to operate. Oxidizing enzyms are the active agents in many cases. Chemical oxidation would intervene only when the temperature had been raised to the combustion point of the substances present. Gore presents formulas and graphs illustrating the theoretical progress of the accumulation of heat.

The studies of E. M. Bailey (*1912*) on the ripening of bananas indicate that bacterial activity is not responsible for the heat produced or other changes resulting during the ripening of the fruit.

Nabokich (*1903*) found that seeds which had been sterilized by chemicals such as mercuric chlorid respired during the first few days as much carbon dioxid as did the controls. In some instances the respiration of the sterilized seeds exceeds that of the controls.

Respiration may be briefly defined as the release of energy through the biochemical oxidation of organic compounds as accelerated by certain enzyms. Carbon dioxid and water are the characteristic chemical end products. It is shown by De Saussure (*1804*) that respiration was accompanied by the disappearance of oxygen. Pflüger (*1875*) maintained that the inspired oxygen combines in some manner with cyanogen radicals of the living protoplasm. This effects a readjustment, as the result of which carbon dioxid and water are eliminated. The decomposition is of an explosive character, and the reaction liberates heat energy.

Verworn (*1899, p. 483*) evolved the "biogen" theory, according to which the oxygen enters the "biogen molecules," which are thus rendered less stable. Slight impulses are then required to bring about a chemical union of this oxygen with the carbon in the cyanogen group.

Since the grain itself is a poor conductor of heat, it follows that the heat energy released through respiration accumulates in the mass in proportion to its bulk so that the increase in temperature may in time become very marked.

MATERIAL OXIDIZED IN RESPIRATION

In the case of resting tissues and storage structures, such as the grain kernel or caryopsis, the exact character of the substrate or material oxidized in respiration is of significance. Wehmer (*1892*) fed Aspergillus on peptone, and found it capable of satisfying its requirements from this source. Gore (*1914*) found that in ripening bananas the rate of starch hydrolysis paralleled the rate of respiration. There was slight change in the quantity of protein and fats in the fruit. Maige and Nicolas (*1910*) found that the immersion of etiolated leaves, shoots, or seedlings in sugar solutions resulted in an increased rate of respiration.

Langworthy and Milner (*1913*) observed that in ripening bananas the "thermal quotient," or quantity of heat produced per unit of carbon dioxid respired, indicated the combustion of carbohydrate. The thermal

quotient found for one period of their studies, when conditions for accurate measurement were at an optimum, was 2.6. This is so nearly the theoretical for the combustion of carbohydrates as to indicate that little else was involved. As pointed out by Dr. Milner in a private communication, the thermal quotient when fat is burned is 3.4, while that when protein is burned is 2.9.

Hasselbring and Hawkins (*1915*) found no general correlation between the total sugar content of the sweet potato and its respiratory activity. Cane sugar is relatively stable and does not appear to be used in the process of respiration. A simultaneous decrease in the reducing sugars and the respiratory activity was observed. The reducing sugars are, in their opinion, the immediate source of respiration material.

That the fat or ether extract of the wheat embryo is not the principal substrate upon which the respiratory enzyms act, and that it is not burned or destroyed during germination is indicated by the experiments of Le Clerc and Breazeale (*1911, p. 12*). Their data show that there was more fat in the total plant (seed residues plus axes) at practically all stages of germination than there was in the original seeds. Accordingly little energy could have been derived from the oxidation of the fats; on the contrary, energy derived from some other source must have been utilized in the synthesis of the additional fat produced in the seedling. All available evidence therefore seems to indicate that the heat of respiration is produced by the oxidation of reducing sugars.

SEAT OF RESPIRATION IN THE WHEAT KERNEL

There are a number of reasons for believing that the germ or embryo of the wheat kernel is the location of the larger part of the biological oxidation that occurs incidental to respiration. The embryo is, in a general way, decidedly richer in enzyms than is the endosperm or any other kernel structure. Brown and Morris (*1890*) consider that the endosperm of the ripened kernel is no longer a vital tissue. The secretion of diastase and other enzyms is assigned to the scutellum, an organ of the embryo. Mann and Harlan (*1915*) concluded that in germinating barley the conversion of the endosperm is effected by enzyms secreted by the epithelial layer of the scutellum.

Karchevski (*1903*) found the energy of carbon-dioxid respiration to be 12 times as great in the wheat embryos as in the seeds themselves. Burlakow (*1898*) states that the respiratory activity of the germ is 20 times greater than is that of the endosperm. Wender (*1905*) called attention to the pronounced catalase activity of the germ structures, as contrasted with mill products derived almost exclusively from the endosperm. This has also been observed in an unpublished study of catalases which was made in this laboratory. This fact is more significant, in view of Appleman's (*1915*) discovery that catalase activity in potato juice shows a striking correlation with respiratory activity in the

tubers. If catalase activity parallels respiratory activity, it may be reasoned by analogy that those structures of the wheat kernel which exhibit the greatest catalase activity are the seat of the larger part of respiration.

Barnes and Grove (*1916*) lend further support to the hypothesis that the seat of oxidation activity is in the embryo by their observation that in air-dry wheat the embryo becomes shrunken after a time, while the food materials of the endosperm are unimpaired. This is interpreted by them to indicate a destruction of the material of the embryo itself as the result of respiration when the dryness of the kernel suppresses diffusion. Were respiration equally pronounced in the endosperm it too should exhibit a similar loss of material.

Osterhout's (*1917*) observation that oxidation is more rapid in the nucleus than in the cytoplasm might lead to the deduction that, since the embryo cells of the wheat caryopsis have a much larger proportion of nuclei than the endosperm cells, oxidation should proceed more rapidly in the embryo tissues.

All of the above facts are in harmony with what might logically be expected. The principal release of energy as the result of biological combustion should occur in the structure where such energy is required for the synthesis of new organic compounds. Since the embryo is endowed particularly with that function, respiration must of necessity be most pronounced in it, if not confined to it.

MEASUREMENT OF THE RATE OF RESPIRATION

There are two general methods which may be used in the quantitative estimation of the rate of respiration of vegetable material. One that is employed where facilities are available is to measure in terms of Calories the heat energy released per unit of time and material. The elaborate device for this purpose is known as the respiration calorimeter. The second general method includes the determination of one of the end products of the reaction, carbon dioxid. This method may easily be made decidedly accurate without entailing the assembling of a calorimeter. In using the calorimeter both carbon dioxid respired and heat evolved may, if desired, be determined simultaneously.

Inasmuch as the writers were not provided with a respiration calorimeter suited to this purpose, the carbon dioxid evolved by stored wheat was measured, and from this data the rate of respiration was calculated. Truog's (*1915*) method and absorption tower was used for this purpose, the tower being slightly modified, or rather, added to, in order to adapt it to the present work. The procedure followed and a description of the apparatus has been published by the junior author (*Gurjar, 1917*). To compute the Calories of heat evolved, the factor found by Langworthy and Milner may be employed; 1 gm. of respired carbon dioxid equals 2.6 Calories of heat.

Calcium-chlorid towers were used as respiration chambers. Paraffined wire netting was fitted into the constriction near the base, and on this the grain rested. Rubber connections were made as short as possible, and all stoppers and tubing were thoroughly paraffined to prevent selective absorption of carbon dioxid. In all instances where the same lot of grain was worked with at different moisture contents, the several portions were brought to approximately the desired percentage of moisture by adding water from a burette, at the same time stirring thoroughly. The samples were allowed to stand in sealed jars for three days, in order to insure uniform distribution of the water through the kernels. It had been found by determining the rate of swelling of the kernels that they reached their maximum size in considerably less than three days, and from this it was concluded that the moisture distribution would be complete in the 3-day interval. When the grain was ready to work with, a weighed quantity was sealed into the tower. Samples were taken at this time for the determination of moisture. The amount of grain placed in the tower varied with the moisture content, about 500 gm. being employed in the case of the lower moisture limits, while about 300 gm. were used when the percentage of moisture exceeded 15.5 per cent. In this manner convenient and accurately determinable quantities of carbon dioxid were obtained. After sealing the grain into the glass cylinders, the air was removed and replaced by carbon-dioxid-free air. The towers were then placed in the thermostat, which, except when temperature was the variable, was maintained at 37.8° C. (100° F.).

The period of incubation was fixed at four days, the exact number of hours being noted at the time of removing the respiration chambers from the thermostat. The accumulated carbon dioxid was then removed through the tubulure at the bottom of the tower, carbon-dioxid-free air being simultaneously admitted through the top. The carbon dioxid was absorbed in *N*/4 barium hydroxid [$Ba(OH)_2$] solution in the special absorption tower, as described by Gurjar in the above-mentioned paper. The respiration data given in the tables are stated in terms of milligrams of carbon dioxid respired per 24 hours by each 100 gm. of dry matter.

RELATION OF THE MOISTURE CONTENT OF WHEAT TO THE RATE OF RESPIRATION

The observation of Bonnier and Mangin (*1885*) that respiration of living plants varies directly with the humidity of the air might be interpreted to mean that the moisture content of the tissues increased in a humid atmosphere. This increase in turn may have occasioned the rise in the rate of respiration. Maquenne (*1900*) concluded that a reduction in the moisture content of seeds is accompanied by a reduction in the rate of respiration, and Lund (*1894*) discovered that the desiccation of roots and tubers reduced their rate of respiration.

Kolkwitz (*1901*) found that barley grains containing 19 to 20 per cent of moisture respired 3.69 mgm. of carbon dioxid per kilo in 24 hours at summer temperature, while at the same temperature barley containing 14 to 15 per cent of moisture respired 1.4 mgm. per kilo, and 0.35 mgm. per kilo when containing 10 to 12 per cent of moisture.

White (*1909*) found that all cereals gave off appreciable quantities of carbon dioxid when stored in an air-dried condition, the respiration of wheat containing 11.9 per cent of moisture being especially pronounced. Wheat dried for eight days at 45° C. did not respire a determinable quantity of carbon dioxid.

Babcock (*1912*) states that respiration is practically suspended in dry seeds and spores, and is most pronounced when vital processes are most active, as during the germination of seeds. The metabolic water produced as respiration proceeds is believed to play an important part in the vital phenomena of the cells.

Duvel (*1904*), in studying the vitality of stored seeds, observed that the rate of respiration, as indicated by the carbon-dioxid content of the air in the closed container, was increased on increasing the moisture content of the seed. At the same time there was a marked diminution in the percentage of viable seed.

Qvam (*1906*) observed an increased rate of respiration in barley as the percentage of moisture was increased.

Duvel (*1909*) held a lot of corn in storage in an elevator bin. The moisture content averaged 17.8 per cent, and the initial temperature on February 17, 1909, was 36° to 40° F., which increased near the surface of the grain to 133° F. on April 27, 1909. The temperature from the middle to the bottom of the bin was only about 40° F. A portion of the hot corn from the top of the bin was artificially dried to an average of 14.57 per cent of moisture. This was put in a car, and as a control a lot of the cool, undried corn from the same bin was put in another car. The latter had an average moisture content of 17.5 per cent. The dried corn remained for 37 days in as good condition as when put in the car, its temperature rising from 57° to 67° F., or a total increase of 10 degrees. The cool, undried corn began to show signs of deterioration in 23 days, and five days later a point near the surface reached a temperature of 122° F. This indicates the increased tendency of the damp grain to heat in storage.

Shanahan, Leighty, and Boerner (*1910*) examined cargoes of American corn on arrival at European ports and observed an increased tendency to heat and "go out of condition" as the moisture content increased.

Duvel and Duval (*1913*) studied the temperature changes in carloads of corn containing different percentages of moisture. In one experiment

running from March 2 to March 29, 1911, the following temperature changes were observed in cars held on track at Baltimore, Maryland:

Moisture.	Average temperature of corn when—	
	Loaded.	Unloaded.
Per cent.	*°F.*	*°F.*
21.6	40.0	109.7
19.9	40.0	41.5
17.4	40.0	40.5
14.1	40.0	41.3

In a similar experiment, running from May 11, 1911, to June 1 and 3, 1911, corn containing 16.9 per cent of moisture or more was heating when unloaded, while that which contained 13.9 per cent of moisture was still cool. The extent of heating bore a fairly definite relation to the shrinkage or loss in weight of the grain.

Bailey (*1917a*) reported to the Second Interstate Cereal Conference in 1916 the results of storage experiments with wheat at Duluth, Minn. In the cool climate of that city it was found that wheat containing 15.5 per cent of moisture when put in a bin in the fall kept 333 days before it developed a sufficiently high temperature to necessitate turning it, while wheat containing 16.5 per cent of moisture was actively heating in 49 days.

The exact reason for an accelerated respiration with an increased moisture content had not been adequately explained. In the discussion referred to in the paper mentioned in the preceding paragraph an hypothesis was advanced to account for this relation. Moisture in grain may, in the light of recent discoveries in the field of physical chemistry, be assumed to exist as imbibed water in loose combination with the organic colloids. The organic colloids which form the principal constituents of the wheat kernel have the property of imbibing considerable quantities of water and forming elastic gels. The gel swells as the water is increased, although the total volume of the dry colloid plus the added water is diminished. The water-imbibing capacity of the several colloids varies widely, starch having an imbibing capacity materially lower than that of wheat gluten. There is no fixed amount which a given dry colloid will imbibe; thus, gels of varying viscosity can be produced, depending upon the proportion of water present, and other variables, such as temperature, mineral salts, and other substances. The rate of diffusion in a gel varies with the viscosity, as pointed out by Plimmer (*1915, p. 386*). In dilute gels diffusion takes place as in water, while in strong gels the rate is slower. It is probable that in very dry grain the imbibed water is not sufficient to produce a gel in the endosperm structures. The colloidal material there located accordingly does not have a

continuous structure, and the possibilities of diffusion are decidedly reduced under such conditions. The exact percentage of moisture below which this discontinuous structure exists in a normal wheat kernel is not known; it probably varies with the percentage of gluten in the grain since gluten possesses a greater water-imbibing capacity than starch. Increasing the moisture content above the maximum at which discontinuity exists results in the formation of a gel through which diffusion

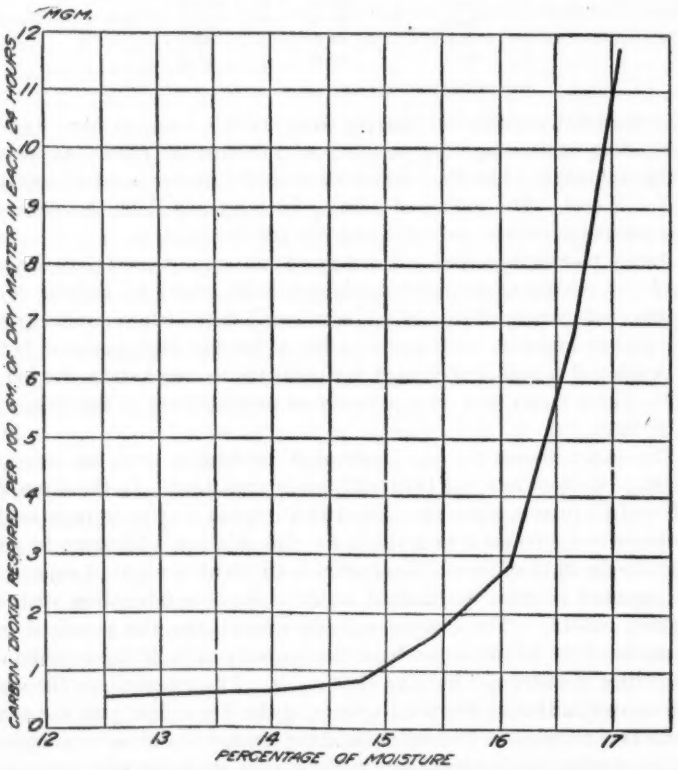

FIG. 1.—Graph showing the relation of the moisture content of wheat to the rate of respiration.

can occur. Further increases in moisture content up to maximum imbibition produce progressively less viscous gels, and correspondingly increase the possible rate of diffusion. Since the rate of respiration in grain doubtless depends in part upon the rate of diffusion between the various kernel structures, it follows that the less viscous the gelatinous material of which the cell contents are composed, the more rapid the production of heat through respiration. To restate, the production of heat is dependent upon the activity of the oxidizing enzyms of the

kernel, the complex phenomenon being known as respiration. The latter is accelerated by an increase in the rate of diffusion, which in turn is dependent upon the existence of a gel, and the relative viscosity of that gel. For these reasons the moisture content of sound grain determines to a considerable extent the rate of respiration and consequent liability of heating when bulk grain is stored.

To determine the relation of moisture content to respiration in stored wheat, a large sample of spring-sown Haynes Bluestem wheat known as Minnesota 169 was obtained. The weight per bushel of the sample was 57½ pounds (26.08 kgm.); the weight per 1,000 average kernels, 24.62 gm.; and it contained 2.21 per cent of nitrogen on the dry basis. It was then divided into several portions, and each portion was brought to a different moisture content, the percentages of moisture ranging from 12.50 to 17.07 per cent. The quantity of carbon dioxid respired per 24 hours by each 100 gm. of dry matter is given in Table I and is shown graphically in figure 1. The rate of increase in respiration is fairly gradual from 12.50 to 14.78 per cent, but after the latter percentage is exceeded the rate is markedly accelerated. The break in the curve occurs when the moisture content slightly exceeds 14.5 per cent, and it is probable that this represents about the maximum percentage of moisture that this class of wheat may safely contain without danger of heating when stored in bulk.

TABLE I.—*Respiration of Haynes Bluestem (Minnesota 169) wheat,[a] incubated at 37.8° C for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
12. 50	0. 54	16. 08	2. 88
13. 93	. 65	16. 65	6. 86
14. 78	. 86	17. 07	11. 72
15. 42	1. 62		

[a] Weight per bushel of sample, 57½ pounds. Weight per 1,000 kernels, 24.62 gm. Nitrogen on dry basis, 2.21 per cent.

The acceleration of respiration as the moisture content increases is shown in Table II. These data are based upon the estimated respiration values at even percentages of moisture, and the computed increase in respired carbon dioxid for each increase of 1 per cent of moisture. In computing these data the following formula was employed: $\frac{K_m - K_{m-1}}{K_{m-1}}$ in which K_m represents the respiration value at a particular percentage of moisture, and K_{m-1} represents the respiration value for the same wheat containing 1 per cent of moisture less than K_m. It is evident that the acceleration between 12 and 14 per cent of moisture is very gradual, while it increases markedly after 14 per cent is exceeded.

TABLE II.—*Acceleration of the rate of respiration of hard spring wheat with increasing moisture content*

Formula.	Acceleration at following percentages of moisture.				
	12 to 13	13 to 14	14 to 15	15 to 16	16 to 17
$\frac{K_m - K_{m-1}}{K_{m-1}}$	0. 16	0. 17	0. 66	1. 41	3. 02

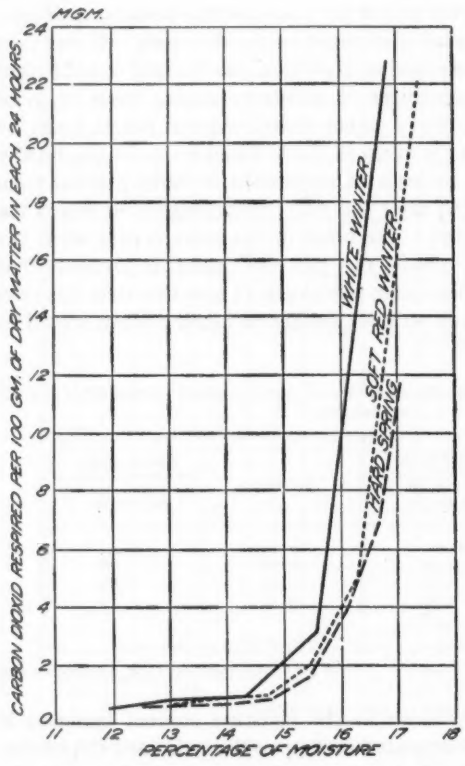

FIG. 2.—Graphs showing the comparative rate of respiration of hard spring, soft red winter, and soft white winter wheat.

RELATION OF THE CONSISTENCY OF THE WHEAT KERNEL TO THE RATE OF RESPIRATION

Reference has already been made to the difference in the relative water-imbibing capacity of the various organic colloids of the wheat kernel. Since starch and gluten constitute a large proportion of the endosperm, their differences in this regard are of principal interest. Simple tests indicate that the water-imbibing capacity of gluten is materially greater than that of starch. In consequence it follows that, as a general rule, those kernels which contain a high percentage of gluten will, at any particular moisture content, be more viscous than will kernels containing a lower percentage of gluten.

The gluten content is also related to the relative consistency or hardness of the wheat berry. In general, the hard, vitreous grains contain a higher percentage of gluten than do the soft, starchy grains. Accordingly we may expect exactly what we find—viz, that the soft wheats are "tougher" and of a lower viscosity at any given moisture content (within the limits found in ordinary commercial grain) than the hard, vitreous wheats.

It is commonly recognized in the grain trade that the keeping qualities of soft wheats are inferior to those of hard wheat. Because of the relation of respiratory activity to rate of diffusion, it should follow that with the same moisture content respiration would proceed more rapidly in a soft than in a hard or vitreous kernel. A sample of soft red winter wheat of the Fultz variety was obtained from the Experiment Station at Columbia, Mo., and another of white winter wheat from Grand Blanc, Mich. The rate of respiration in these soft wheats at different moisture contents was studied, and it was found that, except at the lower percentages of moisture, the rate of respiration was higher in the soft red winter wheat than it was in the hard spring wheat, and still higher in the white winter wheat. These data are given in Tables, III, IV, and V, and graphically in figure 2. As shown by the graph, the curves tend to converge at about 12 per cent of moisture, indicating that at less than this moisture content the discontinuity of endosperm structure referred to above may exist in sound wheats and respiration proceed at the expense of substances in the germ rather than by oxidation of materials which diffuse to it from the endosperm.

It may further be seen that the quantity of heat evolved by hard spring wheat containing 14.5 per cent of moisture, as evidenced by the rate of respiration at that moisture content, was evolved by these soft wheat samples when they contained about 13.6 to 13.8 per cent of moisture. This is of interest, in view of the moisture limits prescribed in the United States Grain Standards for wheat, which are 14½ per cent for No. 2 hard spring, and 13 per cent for No. 2 soft red winter, and the same for common white and white club wheat.

TABLE III.—*Respiration of soft red winter wheat* [a] *from Missouri, incubated at 37.8°C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
13. 07	0. 65	15. 45	2. 00
13. 63	. 80	16. 37	5. 06
14. 70	. 95	17. 40	22. 03

[a] Weight per bushel of sample, 61 pounds. Weight per 1,000 kernels, 29.97 gm. Nitrogen on dry basis, 1.54 per cent.

TABLE IV.—*Respiration of white winter wheat[a] from Michigan, incubated at 37.8°C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
11. 94	0. 48	15. 57	3. 20
13. 04	. 60	16. 83	22. 77
14. 32	. 89		

[a] Weight per bushel of sample, 59 pounds. Weight per 1,000 kernels, 37.73 gm. Nitrogen on dry basis, 1.53 per cent.

TABLE V.—*Interpolated quantity of carbon dioxid respired per unit of time and material, at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Hard spring	0. 50	0. 58	0. 68	1. 13	2. 72	10. 73
Soft red winter		. 63	. 81	1. 37	3. 84	15. 51
White winter	. 49	. 60	. 83	4. 15	9. 85	25. 18

RELATION OF THE RELATIVE PLUMPNESS OF THE WHEAT KERNEL TO THE RATE OF RESPIRATION

It is generally recognized that the velocity of enzym action conforms quite closely to the law of mass action. Thus any condition which affects the quantity of either the substrate or the enzym will cause variations in the rate of the reaction. Since respiration is occasioned by enzyms, the rate of respiration of the wheat kernel should vary with these conditions.

A shriveled condition of the wheat kernel is due generally to factors operating during the later stages of kernel development. The translocation of reserves to the kernel is interfered with by rust, drouth, desiccation by hot winds, or some other agency; and an incomplete filling of the endosperm results. According to Brenchley (*1909*), the germ portion of the kernel is developed earlier than the endosperm, and tends to escape injury from the agency causing shrunkenness of the endosperm more than does the latter. The diminished size and weight of the shriveled kernel is therefore due principally to the decreased quantity of endosperm.

The enzymic activities of the kernel seem to be mainly invested in the embryo. This was discussed in one of the foregoing paragraphs. In the embryo of the shriveled wheat berry the enzyms are probably represented practically as they are in the normal kernel. In instances where the average weight of the individual kernel is only about half the normal,

it follows that there is approximately twice the enzymic activity per unit of mass than that shown by normal wheat. The normal spring wheat used by the writers weighed 24.62 gm. per 1,000 average kernels. A shriveled sample of the same type of wheat was obtained which weighed 11.73 gm. per 1,000 kernels, or less than half the weight of the normal. The rate of respiration of these two lots was compared, and, as shown in figure 3, the respiratory activity of the shriveled sample decidedly exceeded that of the normal, or plump wheat. Thus the quantity of carbon dioxid respired by the latter when it contained 14.5 per cent of moisture was respired by the shriveled wheat used when it contained only 12.8 per cent of moisture. The curves tend to converge at moisture contents slightly below 12 per cent.

The respiration data for the shriveled sample are given in Table VI, while in Table VII is shown the interpolated quantity of carbon dioxid respired by the normal and shriveled wheats at even percentages of moisture.

TABLE VI.—*Respiration of shriveled spring wheat,*[a] *incubated at 37.8° C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
12. 68	0. 75	15. 68	4. 50
13. 19	. 94	16. 09	10. 51
14. 29	1. 38	16. 44	16. 92
15. 30	3. 02	16. 80	21. 65

[a] Weight per bushel of sample, 47¼ pounds. Weight per 1,000 kernels, 11.73 gm. Nitrogen on dry basis, 2.03 per cent.

TABLE VII.—*Interpolated quantity of carbon dioxid respired per unit of time and material at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Plump spring wheat.....	0. 50	0. 58	0. 68	1. 13	2. 72	10. 93
Shriveled spring wheat.....	. 65	. 88	1. 26	2. 54	9. 41	22. 65

SOUNDNESS OF THE WHEAT KERNEL IN ITS RELATION TO THE RATE OF RESPIRATION.

A form of unsoundness recognized as such by the grain trade and frequently occurring in spring wheat is the frosted condition. This results from the freezing of the plants before the grain is matured and desiccated. The plants usually thaw later, and, while the protoplasm

is killed or disorganized and its synthetic activities reduced or destroyed, certain hydrolytic enzyms are activated, and hydrolysis or splitting of certain of the kernel constituents ensues. As a result, there is an accumulation of the split products of starch and proteins, particularly dextrose and amino acids. It is probable that the extent of starch hydrolysis by amylases depends in large part upon the percentage of moisture in the grain after it thaws out. If the kernels are nearly dry, less change will occur than if the kernels contain considerable moisture.

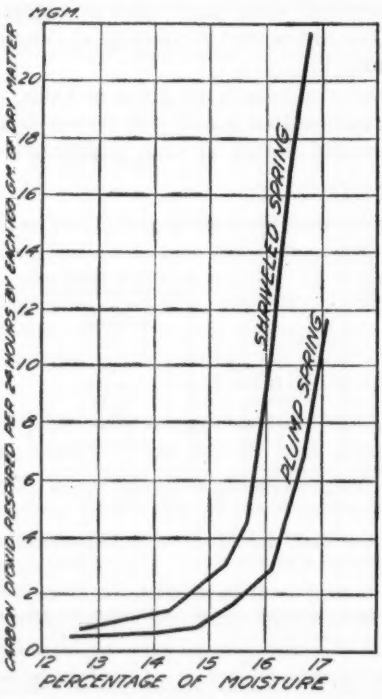

FIG. 3.—Graphs showing the rate of respiration of shriveled wheat and of plump wheat of the same class.

The dextrose which thus accumulates in the kernel is presumably available as substrate for the respiratory enzyms. In accordance with the law of mass action, a greater concentration of substrate should accelerate the rate of respiration. There is an additional factor in the case of frosted wheat that would also tend to result in an increased rate of respiration at any particular percentage of moisture. The hydrolysis of the gluten subsequent to thawing results in products having a materially lower water-imbibing capacity than the normal gluten. In fact, the amino acids formed are not colloids, and form true solutions. Consequently the relative viscosity of frosted grain at any moisture content will be less than in normal grain, the difference depending upon the extent of hydrolysis.

To ascertain the effect of frosting upon the rate of respiration, two samples of commercial wheat containing frosted kernels were secured. These were marked "moderately frosted" and "badly frosted," respectively. The respiratory activity of these frosted samples was determined with five different percentages of moisture present. Tables VIII and IX show the respiration data of these two lots, while in Table X are given the interpolated values at even percentages of moisture in comparison with sound spring wheat. The same data are shown graphically in figure 4. There is a slight overlapping of the curves for the

moderately and badly frosted samples between 14.5 and 15.5 per cent of moisture, the reason for which is not clear. The discrepancy is not great, and the similarity of the curves indicates that possibly our judgment was at fault in describing the two lots of frosted wheat as "moderately" and "badly" damaged. The decided differences in the rate of

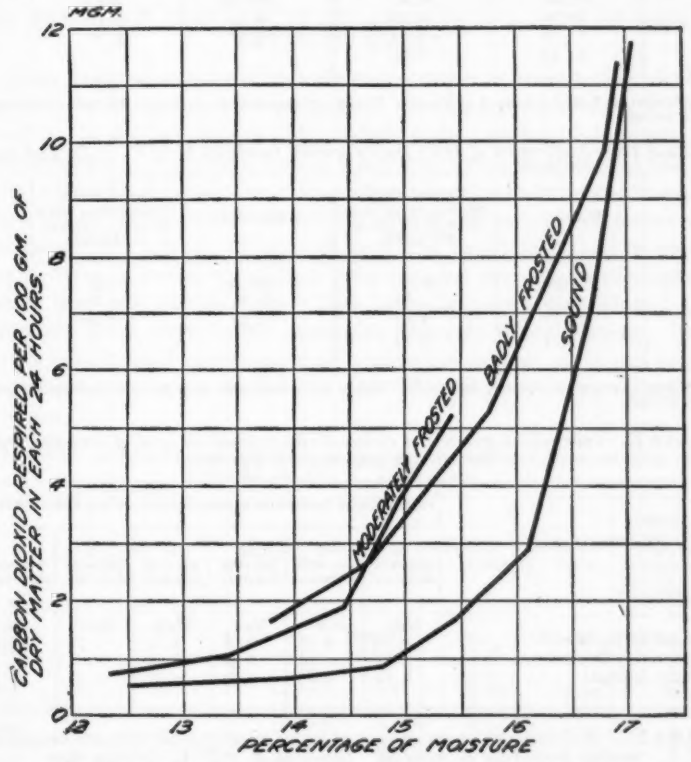

FIG. 4.—Graphs showing the rate of respiration of frosted wheat and sound wheat of the same class.

respiration of frosted and of normal wheat containing up to 16.5 per cent of moisture substantiates what had previously been empirically observed by the senior author (*Bailey, 1917b*)—viz, that frosted wheat tends to heat more readily when stored than does sound wheat containing the same percentage of moisture.

TABLE VIII.—*Respiration of moderately frosted wheat,[a] incubated at 37.8° C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
12. 32	0. 74	14. 95	3. 75
13. 38	1. 04	15. 42	5. 21
14. 44	1. 89		

[a] Weight per bushel of sample, 58 pounds. Weight per 1,000 kernels, 36.94 gm. Nitrogen on dry basis, 2.02 per cent.

TABLE IX.—*Respiration of badly frosted wheat,[a] incubated at 37.8° C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
13. 79	1. 63	16. 81	9. 82
14. 67	2. 64	16. 95	11. 40
15. 74	5. 24		

[a] Weight per bushel of sample, 53 pounds. Weight per 1,000 kernels, 22.52 gm. Nitrogen on dry basis, 2.10 per cent.

TABLE X.—*Interpolated quantity of carbon dioxid respired per unit of time and material, at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Sound spring wheat	0. 50	0. 58	0. 68	1. 13	2. 72	10. 93
Moderately frosted	. 65	. 94	1. 52	3. 90	.....	.....
Badly frosted	1. 12	1. 20	1. 87	3. 46	6. 35	11. 97

TABLE XI.—*Respiration of several samples of frosted wheat from car lots, and containing varying percentages of moisture. Incubated at 37.8° C. for four days*

Laboratory No.	Moisture.	Weight per bushel.	Weight per 1,000 kernels.	Nitrogen on dry basis.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Interpolated respiration of sound wheat of same water content.
	Per cent.	*Pounds.*	*Gm.*	*Per cent.*	*Mgm.*	*Mgm.*
G122	14. 30	60¼	28. 36	2. 23	1. 71	1. 30
G123	14. 82	61	28. 64	2. 20	2. 34	1. 73
G118	16. 16	56½	23. 56	2. 27	8. 71	6. 86
G102	16. 19	57	28. 88	2. 00	8. 24	7. 15

Several wheat samples taken from car lots by samplers of the State Grain Inspection Department were frosted, and the respiratory activity

of four of these was measured. In every case the rate of respiration was greater than in sound wheat of the same moisture content which was taken from car lots at the same time. The tests of these frosted samples are shown in Table XI, the last column of which shows the interpolated respiration data of sound wheat.

THE PERIOD OF DAMPNESS AS INFLUENCING THE RATE OF RESPIRATION OF WHEAT

When damp wheats are stored, the excess moisture which is responsible for their dampness has been present for varying lengths of time. This is called for the purposes of this discussion the "period of dampness."

In connection with these studies a series of wheat samples was taken from car lots by the State Grain Inspection Department between the 15th and 27th of March, which contained varying percentages of moisture. The respiratory activity of each of these was determined, and on plotting a curve it was observed that these samples gave materially higher values than the samples which had been dampened and allowed to stand for three days before measuring the rate of respiration. The curves tend toward convergence at a moisture content of 12 per cent. These data are given in Tables XII and XIII and are shown graphically in figure 5.

TABLE XII.—*Respiration of natural hard spring wheat collected from car lots, and incubated at 37.8° C. for four days*

Laboratory No.	Moisture.	Weight per bushel.	Weight per 1,000 kernels.	Nitrogen in dry matter.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
	Per cent.	*Pounds.*	*Gm.*	*Per cent.*	*Mgm.*
G128	12.47	62	30.56	2.14	0.61
G127	13.11	63	32.94	2.16	.75
G124	14.70	58½	24.60	2.05	1.49
G120	15.51	52	26.92	2.31	3.26
G104	15.73	59	28.64	2.47	3.94
G103	16.00	59	25.72	2.37	5.69
G107	16.53	56	22.88	2.25	10.65
G109	16.90	51	.....	.....	13.86

TABLE XIII.—*Interpolated quantity of carbon dioxid respired per unit of time and material, at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Freshly dampened wheat	0.50	0.58	0.68	1.13	2.72	10.73
Natural wheat	.51	.73	1.15	2.14	5.69	15.03

Since the principal difference between the samples dampened by the writers and the damp grain obtained from the freight cars was the length of the period of dampness, an experiment was conducted to ascertain the extent to which that variable affected the rate of respiration. Two samples of Bluestem wheat that had been dampened some time previous

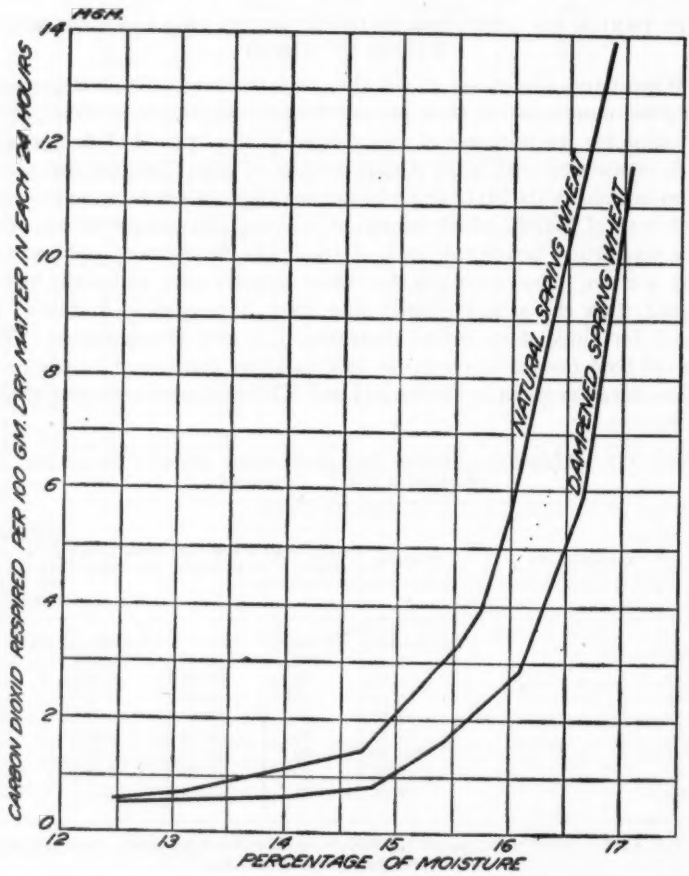

FIG. 5.—Graphs showing the comparative respiratory activity of naturally damp wheats and of wheats dampened in the laboratory three days before they were incubated.

and stored at a temperature of about 25° C. in the laboratory vault were incubated in the usual manner at 37.8° C. with the results shown in Table XIV. The quantity of carbon dioxid respired by the sample containing 15.21 per cent of moisture, which was stored for 55 days after it was dampened, was about four times as great as that respired by freshly dampened wheat of the same moisture content, while that from

the sample containing 15.71 per cent of moisture, which was stored for 108 days, was about eight times as great as for freshly dampened wheat containing that percentage of moisture.

TABLE XIV.—*Respiration of dampened wheat after storage at about 25° C. Incubated at 37.8° C. for four days*

	Lot A.	Lot B.
Moisture.....per cent..	15.21	15.72
Number of days stored.....	55	108
Carbon dioxid respired per 100 gm. of dry matter in each 24 hours.....mgm..	5.31	17.00
Carbon dioxid respired per 100 gm. of dry matter in each 24 hours, of same lot of wheat 4 days after dampening.....mgm..	1.35	2.17
Carbon dioxid respired by naturally damp wheat from car lots, of same moisture content.....mgm..	2.59	3.85

Table XIV also shows a decided difference in the comparative rate of respiration of wheat stored for a time in a warm room and that obtained from freight cars. The latter had no doubt been damp for a longer time than that which was dampened and held in the room. These data suggest that not only does the period of dampness affect respiration, but the conditions of storage may have an equally important effect. The grain taken from cars was cold at the time, and had probably been exposed to the cold atmospheric conditions of the preceding winter months. If, as postulated by the writers, dextrose tends to accumulate in the stored damp grain the rate of accumulation would depend upon the temperature of the grain as well as upon the time elapsed. Accordingly there may have been more substrate (dextrose) for the respiratory enzyms in the grain which had been stored in the warm room than there was in that stored out of doors during the winter months. A series of experiments with both the period of dampness and the temperature of storage as variables have accordingly been begun, and the results will be reported later in another publication.

INFLUENCE OF TEMPERATURE ON THE RESPIRATION OF STORED WHEAT

Pfeffer (*1878*) observed that the intensity of respiration increases with the temperature until the latter begins to injure all the vital processes. Hoff (*1896*, p. *125*) stated that the rate of respiration increases two or three times for each 10-degree rise in temperature in accordance with the usual rule of chemical reactions. Ziegenbein (*1893*) found that temperatures above 45° C. were injurious

Clausen (*1890*) studied the respiration of germinating wheat at different temperatures and found the optimum to be about 40° C. The rate of respiration was 2.86 times as great at 10° as at 0° and 1.09 times as great

at 40° as at 30°. The average increase between 0° and 40° was 2.71 times for each 10-degree increase in temperature.

Qvam (*1906*) found the rate of respiration increased up to at least 45° C., which was apparently the highest temperature at which observations were made. The grain was very moist, 100 gm. of water having been added to 200 gm. of grain.

Matthaei (*1905*) investigated the respiration of cherry-laurel leaves at different temperatures and reported an increasing rate of respiration between 5.8° and 33.1° C. At 5.8° 2 gm. of green leaves respired 0.1 mgm. of carbon dioxid per hour, and at 33.1° the rate was 1.35 mgm. per hour.

The experiments of Duvel and Duval (*1913*) with shelled corn indicate the relation of air temperatures to the heating of such material. Shelled corn in transit and on track containing 16.9 per cent of moisture began to heat and go out of condition between May 11 and June 3, 1911, while in the period from December 24, 1910, to January 20, 1911, corn containing 22 per cent of moisture gained only a few degrees in temperature. The purely physical factor of heat loss into the cold winter atmosphere, of course, served to reduce the rate of rise in temperature, but there was probably a diminished rate of evolution of heat as well.

Attention has been called by Bailey (*1917a*), to the influence of atmospheric temperatures upon the rate of heating of damp wheat. A lot of wheat containing 16.5 per cent of moisture required but 11 days to increase in temperature from 70° to 80° F., when the mean air temperature was 62.1° F., while later in the year, when the mean air temperature was 44.3° F., another lot of wheat containing the same percentage of moisture was stored 49 days before its temperature increased to the same extent.

Another comparison of the influence of temperature on the rate of heating in storage was afforded by two lots of wheat put into bins at about the same time and containing nearly the same percentage of moisture. The initial temperature of one lot was 74° F., and of the other 70°. The latter required over five times as long to reach a temperature of 80° as did the former, owing to the slow increase in temperature at the outset as contrasted with the rapid rate of increase as the temperature mounted higher.

To ascertain the relation of temperature to the rate of respiration in stored grain, a large sample of Minnesota 169 Bluestem wheat was dampened until it contained 14.96 per cent of moisture. Aliquots of this sample were sealed in glass jars and kept in a refrigerator until they were used. This was done to minimize enzymic changes in the grain. The necessary quantities were drawn from the refrigerator for incubation at the several temperatures.

The lowest temperature at which observations were made was 4° C., and since the increase in rate of respiration between 4° and 25° was rela-

tively small, no intermediate temperatures were employed. Regular increases in the temperature of the thermostat by 10-degree intervals were then made until the respired carbon dioxid showed a marked diminution.

The data in Table XV and the graph in figure 6 show that the rate of respiration increased to a maximum at 55° C. This is therefore the temperature at which the most rapid evolution of heat would occur. A discoloration of the seed coat of the wheat kernel begins to show on some kernels at about 35° C., while at 55° the whole mass is of a mahogany color. At 65° the respiratory enzyms have been partially but not

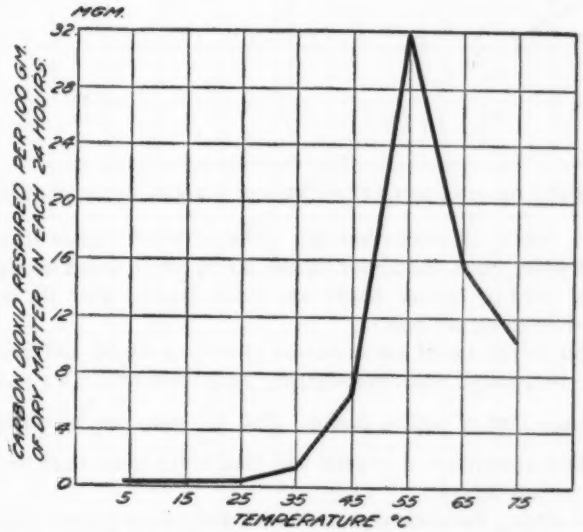

FIG. 6.—Graph showing the relation of temperature to the rate of respiration.

wholly inactivated, while at 75° this inactivation has proceeded still further, and some roasting of the grain has occurred.

TABLE XV.—*Respiration of hard spring wheat at different temperatures*

Temperature.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Temperature.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
°C.	*Mgm.*	°C.	*Mgm.*
4	0.24	55	31.73
25	.45	65	15.71
35	1.30	75	[a] 10.28
45	6.61		

[a] A part of this carbon dioxid may have resulted from roasting the grain.

The proportional change in respiration for each 10-degree rise in temperature is shown in Table XVI. The data in this table were obtained by employing the conventional formula $\frac{V_{t+10}}{V_t}$ in which V_t represents the rate of respiration at the specified temperature, and V_{t+10} represents the rate at a temperature 10 degrees higher. The values at 5° and 15° were computed by integrating the actual data obtained at 4° to 25° and 35° C.

TABLE XVI.—*Acceleration of respiration for each 10-degree increase in temperature*

t.	$\frac{V_{t+10}}{V_t}$	t.	$\frac{V_{t+10}}{V_t}$
° C.		° C.	
5	1. 16	35	5. 08
15	1. 55	45	4. 80
25	2. 89	55	. 49

INFLUENCE OF ACCUMULATED CARBON DIOXID UPON RESPIRATION

Müntz (*1881*) observed that ten times as much carbon dioxid was respired when grain had access to free air as when sealed air-tight and that the yield of carbon dioxid was much greater after the moisture exceeded 13 to 14 per cent.

Mangin (*1896*) found the evolution of carbon dioxid and absorption of O_2 to be reduced when germinating seeds were put in air containing up to 5 per cent of carbon dioxid. The $\frac{CO_2}{O_2}$ ratio was increased, indicating that absorption of oxygen was diminished more than the evolution of carbon dioxid.

Duvel (*1904*), Babcock (*1912*), Barnes and Grove (*1916*), and others have called attention to the reduced vitality and germination of seeds stored in carbon dioxid, or in tight containers in which the respired carbon dioxid accumulated.

In the case of grain stored under ordinary commercial conditions it follows that the oxygen in the space surrounding the kernels must be replaced with respired carbon dioxid. The rate of such replacement will, of course, hinge upon the factors influencing the rate of respiration. To determine the relative change in the respiration of grain stored in a tight container in which the respired carbon dioxid must accumulate, the following experiment was conducted: A sample of wheat containing 15.05 per cent of moisture was divided into four portions, which were incubated in the usual manner at 37.8° C. At the end of 1, 4, 8, and 12 days a cylinder was removed and the respired carbon dioxid determined. The rate of respiration for the first day and the average rate for each of the 4-day periods are shown in Table XVII and graph-

ically in figure 7. Both table and graph show plainly that the rate of respiration is reduced by the accumulated carbon dioxid, and it is probable that a further reduction in respiratory activity would result if the proportion of carbon dioxid were increased. This fact has an important bearing on commercial practices. It indicates that stored grain should not be disturbed so long as its temperature does not exceed that of the atmosphere, since exposing it would serve to ventilate the grain and thus

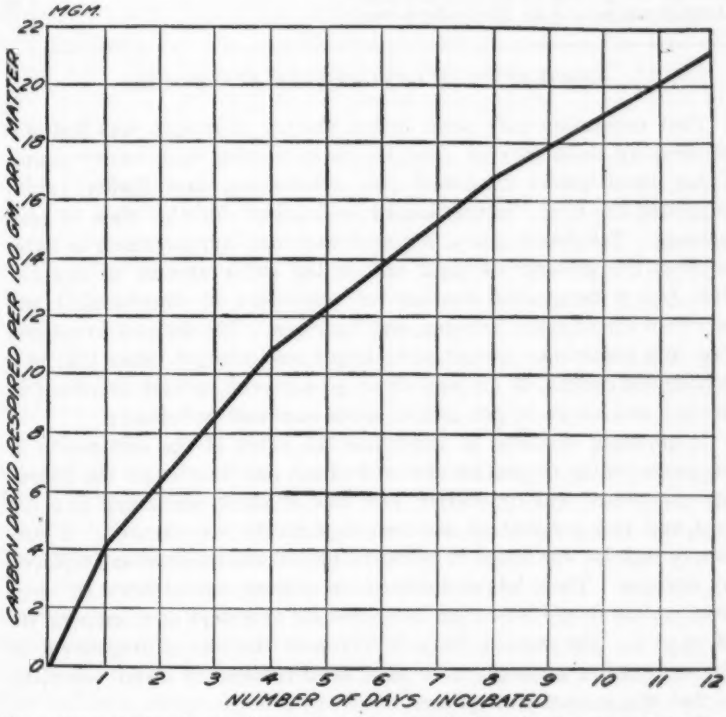

FIG. 7.—Graph showing the rate of respiration during successive intervals when the respired carbon dioxid was permitted to accumulate in the mass of grain.

remove the carbon dioxid. On returning such grain to the bin after aerating it the rate of respiration would be increased over that of the grain which was not handled and exposed, since the rate of respiration of the unaerated grain has been depressed, owing to the accumulated carbon dioxid. The aerated grain will accordingly heat more rapidly than before aerating, unless its temperature has been materially reduced by exposing it to the air.

TABLE XVII.—*Rate of respiration per day for several successive periods*

Period.	Carbon dioxid respired per 100 gm. of dry matter.
	Mgm.
First day	4. 11
Average rate per day for first 4-day period	2. 68
Average rate per day for second 4-day period	1. 49
Average rate per day for third 4-day period	1. 11

RESPIRATION IN OXYGEN-FREE ATMOSPHERE

That respiration may occur in the absence of oxygen was first discovered by Rollo in 1798 (*Hill, 1913*) in working with barley grains. Other investigators confirmed this observation, and Pfeffer (*1878*) suggested the term "intramolecular respiration" for this class of phenomena. Takahashi (*1905*) reported that rice can germinate in water without the presence of sugar and in the entire absence of any air. Hill (*1913*) determined the rate of respiration of water-soaked and sterilized wheat in air, nitrogen, and hydrogen. The decrease in respiration in a continuous current of hydrogen and nitrogen below that in a continuous current of air was about 50 per cent in seeds sterilized in alcohol and about 80 per cent in seeds sterilized in formalin.

It appeared desirable to determine the effect of the elimination of oxygen upon the respiration of stored wheat, and to this end the following experiment was conducted: Two lots of wheat containing 15.6 per cent and 17.6 per cent of moisture respectively were secured. A portion of each lot was sealed in cylinders, the air was removed and replaced by nitrogen. These lots and controls in ordinary atmospheric air freed from carbon dioxid were then incubated for four days at a temperature of 23.9° C. The data in Table XVIII show the rate of respiration in the oxygen-free atmosphere to have been reduced to about two-fifths of that in a normal atmosphere.

TABLE XVIII.—*Comparative rate of respiration in oxygen-free and normal atmosphere*

Moisture.	Carbon dioxid respired per 100 gm. of dry matter in each 24 hours.	
	Oxygen-free atmosphere.	Normal atmosphere.
Per cent.	*Mgm.*	*Mgm.*
15. 6	0. 43	1. 10
17. 6	2. 80	6. 80

CONCLUSIONS

(1) Deductions from these investigations support the findings of earlier investigators that spontaneous heating in damp grain is occasioned by the biological oxidation of dextrose and similar sugars, chiefly in the germ or embryo of the kernel.

(2) Moisture is one of the determining factors in respiration. It establishes the comparative rate of diffusion between the several kernel structures. Any gain in the moisture content of the kernel accordingly increases the rate of diffusion and, simultaneously, the rate of respiration. The increase is gradual and fairly uniform until the moisture exceeds 14.5 per cent, in the case of plump spring wheat, when it is markedly accelerated.

(3) Density of the wheat kernel generally parallels the gluten content. Gluten possesses the property of imbibing more water than starch, and thus varying percentages of gluten result in varying degrees of viscosity at the same moisture content. The relative viscosity affects the rate of diffusion and this in turn directly affects the rate of respiration. The soft, starchy wheats thus respire more rapidly than hard, vitreous wheats containing the same percentage of moisture.

(4) Plumpness of the wheat kernel affects the rate of respiration, as shown by contrasting plump and shriveled grain. The shriveled wheat respired two to three times as much as did the plump wheat at moisture contents above 14 per cent. At percentages of moisture below 14 per cent the difference is not very marked. The high acceleration of respiration in shriveled wheat containing more than 14 per cent of moisture is attributed to the higher ratio of germ to endosperm and hence the larger percentage of enzym to substrate as compared with plump wheat.

(5) The period of dampness—that is, the length of time the excess moisture has been present in the wheat—bears a relation to the rate of respiration. This is shown by comparing the respiration of freshly dampened wheat with that of naturally damp grain and with grain that had been dampened and stored for varying lengths of time. The curve of respiration diverges from that of freshly dampened wheat when the moisture content exceeds 12 per cent, and this divergence is more marked after 13 per cent of moisture is reached. In the case of wheat dampened and stored, the quantity of carbon dioxid respired varies directly with the number of days the wheat remained in storage. The temperature at which the grain is stored affects the rate of diastatic action, thus increasing the quantity of substrate available to the respiratory enzyms. This is indicated by the greater rate of respiration of wheat stored at room temperature than that stored at the outdoor temperature during the winter months.

(6) Unsoundness of wheat caused by the freezing of the unripe plant results in higher respiratory activity in the threshed grain. This was

shown by comparing moderately and badly frosted wheats with sound wheat. The frosted wheat respired more vigorously than the sound wheat. This was attributed to the arresting of the synthetic processes on freezing, and subsequent activities of the hydrolytic enzyms on thawing of the frozen wheat. The accumulation of glucose as the result of starch hydrolysis furnishes larger quantities of substrate to the respiratory enzyms.

(7) Increasing temperatures accelerate the rate of respiration until 55° C. is reached. As the temperature rises the diastatic action upon starch increases. A point is reached, however, at which the enzym activity diminishes.

(8) Accumulation of carbon dioxid in the respiration chamber decreases the rate of respiration. The mean rate by four-day intervals is highest for the first four days and diminishes materially in successive periods.

(9) Respiration is reduced in an oxygen-free atmosphere, the ratio to that occurring in a normal atmosphere being about 1 to 2.5.

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EFFECTS OF MISTLETOE ON YOUNG CONIFERS

By JAMES R. WEIR

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During the years 1911 to 1917 the writer has been engaged in the investigation of the injurious effects of mistletoes of the genus Razoumofskya (Arceuthobium) on conifers. A part of these investigations have already been published.[1] During the course of the work, a number of studies were made which have not yet been reported. It seems that these studies are of sufficient importance to be presented at this time.

The fact that these parasites are a cause of suppression [2] in forest trees is readily appreciated after the injury has become acute. In middle-aged and older trees where accumulated injury has resulted in small diameters and broomed scraggly crowns with reduced leaf surface, the effect of the parasites is quite evident (Pl. 37, A). The effects of mistletoe on its host is by no means so apparent on trees ranging in age from 4 to 10 years. Trees which have become infected early in life may begin to react in marked degree, but the extent of the suppression is not always apparent to the eye. It may be noticed that the branches of young trees are broomed along with other types of infection of a more general nature, but the retardation of the excurrent or elongated growth of the main stem may not be readily recognized. This is where detailed measurements are of value and prove or disprove the early suppression of trees of the younger age classes by mistletoe.

In order to demonstrate the suppression in young trees, a series of measurements of the height growth of western yellow pine (*Pinus ponderosa*) were made in Spokane County, Washington. Two representative plots of 1 acre each, consisting of infected and uninfected reproduction and representing all age classes, were selected. The site was level bench land with a sparse stand of merchantable-sized trees. Some of these trees were severely infected with mistletoe [*Razoumofskya campylopoda*

[1] WEIR, J. R. LARCH MISTLETOE: SOME ECONOMIC CONSIDERATIONS OF ITS INJURIOUS EFFECTS. U. S. Dept. Agr. Bul. 317, 25 p., 13 fig. 1916.

——— MISTLETOE INJURY TO CONIFERS IN THE NORTHWEST. U. S. Dept. Agr. Bul. 360, 39 p., 27 fig. 1916. Literature cited, p. 39.

——— SOME SUGGESTIONS ON THE CONTROL OF MISTLETOE IN THE NATIONAL FORESTS OF THE NORTHWEST. In Forestry Quart., v. 14, no. 4, p. 567–577. 1916.

[2] The term "suppression" is not used here in the ordinary forestry sense, but refers to a retardation of growth induced by parasitic organisms.

(Engelm.) Piper] and were the source of infection of the reproduction. The young trees were well distributed over the area and were dominant.

The results of this study are summarized in Table I and demonstrate very clearly the damage which may result from attack by this parasite on yellow pine.

TABLE I.—*Effect of mistletoe on yellow pine*

Age of tree.	Basis No.		Average height of trees.		Difference in height between infected and uninfected trees.
	Infected.	Uninfected.	Infected.	Uninfected.	
Years.			*Cm.*	*Cm.*	*Cm.*
4	8	50	15.22	25.38	10.15
5	10	27	22.84	35.53	12.69
6	14	42	35.53	50.76	15.22
7	9	35	48.22	71.06	22.84
8	19	20	53.29	78.68	25.38
9	10	23	63.45	93.90	30.45
10	7	16	73.60	109.13	35.53
Total	77	213	44.72	57.87	13.15

During the above study it was plainly observed that the length of the internodes of the infected trees, also the length of the terminal and lateral buds of the main shoot, were much shorter than those of the uninfected, and led to the following study on Douglas fir (*Pseudotsuga taxifolia*) in the Missoula River region near Missoula, Mont. The trees were infected with *Razoumofskya douglasii* Engelm. For this study trees were selected over an area without recourse to sample plots. The only point adhered to was the selection of trees on same type of site, condition of growth, and an average age of 18 years. The results are given in Table II

TABLE II.—*Effect of mistletoe on Douglas fir*

Trees seriously infected.										Trees vigorous, not infected.									
Basis (number of trees).	Average height growth of last four internodes.				Average dimension terminal bud.		Average dimension and number of lateral terminal buds.			Basis (number of trees).	Average height growth of last four internodes.				Average dimension terminal bud.		Average dimension and number of lateral terminal buds.		
	1	2	3	4	Length.	Breadth.	No.	Length.	Breadth.		1	2	3	4	Length.	Breadth.	No.	Length.	Breadth.
	Cm.	*Cm.*	*Cm.*	*Cm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*		*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*
50	28	23	23	18	8	3	108	5	3	50	36	33	36	35	12	4	157	7	3+

The foregoing results clearly demonstrate the effect of the formation of brooms and burls on the storage of food materials in the terminal buds and shoots. It is a well-known fact that in the terminal bud are stored

the elaborated food materials for its early development the following season. If this food material is reduced in amount by its becoming localized in other parts of the tree, the growth of the main shoot must be necessarily retarded (Pl. 37, B), and the bud itself will form earlier in the season and be much reduced in size. Two yellow-pine trees, each 8 years of age, one with a conspicuous infection with broom formation, the other entirely free from infection, were carefully observed to determine this point. The former not only started the elongation of the main shoot nine days later than the other tree, but ceased to develop altogether at the end of the first month. The shoot of the uninfected tree continued to elongate for two months and showed a gain of 11 inches over that of the infected tree. The trees grew under exactly similar conditions, and their root systems were practically equal in extent. Kirkwood[3] states that—

> It is improbable that the whole growth of the new leader is at the expense of the food stored in the bud alone. That from other parts also doubtless contributes, but the tendency is to crowd the formative materials toward the extrmities of the main shoot and the branch. In the sharing of these materials the main shoot leads and the branches follow in order of their importance. The principal growth, however, is undoubtedly at the expense of the locally stored materials, the substances stored elsewhere having their part to play in the development of the tissues in their immediate proximity.

The storage of food in the shoot and branches is exactly what the formation of brooms and burls prevents in a large measure in all parts of the tree above the seat of infection, and eventually results in retardation and the appearance of spike top or staghead. The writer has repeatedly called attention to this in previous publications. In order to demonstrate that there is an actual storage of food materials in mistletoe brooms greater than that of normal branches the results of an experiment may be given. Late in the month of October, 1914, after the leaves had fallen, 10 mistletoe brooms and 10 uninfected branches from points ranging from 5 to 15 feet above the former were cut from a western larch and thrown in a damp, shady ravine. In June of the following year the brooms and branches were examined with the following result: Practically all foliar spurs of the brooms developed needles in proportion of about one-third of the normal length. A few of the foliar spurs of the uninfected branches produced needles barely protruding from the bud scales, and in most cases there was no leaf production whatever. Observation of brooms in the crowns of larch cut late in the fall during logging operations showed in the production of needles in the spring that there must be a great localization of elaborated food materials in the branches of the brooms over that of the normal branches.[4] The latter showed no signs of foliation.

[3] KIRKWOOD, J. E. THE INFLUENCE OF PRECEEDING SEASONS ON THE GROWTH OF YELLOW PINE. *In* Torreya, v. 14, no. 7, p. 118. 1914.

[4] Certain parasitic fungi also cause a flow of building materials to the place of infection. See GOEBEL, K. E. EINLEITUNG IN DIE EXPERIMENTELLE MORPHOLOGIE DER PFLANZEN. p. 75. Leipzig und Berlin, 1908.

From the foregoing studies it may be concluded that the false mistletoes are serious agents in the suppression of young forest growth. Moreover, young growth once infected on the main stem (and it is usually so infected) can not recover and produce merchantable material. Suppression in young yellow pine even up to the sapling stage is of serious consequence. Very seldom do such trees ever overcome the early influence. This is also noticeable in trees of dense stands or when overtopped by older classes. The ultimate effect on the growth of the tree is exactly equivalent to lack of light, only worse. In the former case the excurrent growth is arrested, and the tree either develops into one continuous broom or dies; in the latter, a sapling of some merchantable value may result. It is clear that every effort should be made in regions of heavy mistletoe infection ro reduce the infection of reproduction by cutting all infected overtopping trees. Care should be taken to prevent the introduction of mistletoe-infected transplants in regions where the parasites do not occur.[5] Such regions, for example, are the Black Hills of South Dakota and several of the southeastern Montana forests.

SUMMARY

The height growth of young trees is greatly retarded by mistletoe. The effects to be observed are reduction in the length of the internodes, small dimensions of the terminal buds, and reduction in number. This result is caused by the localization of food materials at the seat of infection.

To reduce the chances of infecting young growth, all overtopping infected trees should be killed. Infected trees of any age should be killed if possible. Care should be taken that infected trees are not planted in regions where mistletoe does not occur.

PLATE 37

A.—*Pseudotsuga taxifolia* infected with *Razoumofskya douglasii*.

B.—Effect of an inoculation with *Razoumofskya campylopoda* on the height growth of 6-year-old *Pinus jeffreyi*. Culture made in a greenhouse at Missoula, Mont.

[5] WEIR, J. R. MISTLETOE INJURY TO CONIFERS IN THE NORTHWEST. U. S. Dept. Agr. Bul. 360, 39 p. 27 fig. 1916. Literature cited, p. 39.

A

B

DETERMINATION OF FATTY ACIDS IN BUTTER FAT: I[1]

By E. B. HOLLAND, *Associate Chemist*, and J. P. BUCKLEY, Jr., *Assistant Chemist*, *Massachusetts Agricultural Experiment Station*

INTRODUCTION

Since reporting a method (*14*)[2] for the determination of stearic acid in butter fat, work has been continued with a view of evolving a process for determining some of the remaining fatty acids. The object of the investigation was to deduce practical analytical methods which might serve to measure the effect of feed upon the composition of the resulting butter fat. Such methods, however, if quantitative and reasonably workable, would be applicable to other fixed oils and fats and play a part in the so-called technical examination of such products.

There are several distinct lines of procedure upon which methods for determining different fatty acids in a mixture might be based:

(1) Crystallization of the acids;
(2) Solubility of various salts;
(3) Fractionation of the acids *in vacuo;*
(4) Fractionation of methyl or ethyl esters.

The iodin absorption and the acetyl substitution are measures of unsaturated and of hydroxy acids, respectively. They are valuable adjuncts, but of limited rather than of general application.

Attention has been called in several instances to some of the inherent faults (*13*, *14*) of the different schemes of separation, and a discussion of their relative merits appears unnecessary at this time. It will suffice to summarize the present application and limitation of the schemes.

Crystallization methods have been employed for the quantitative separation of stearic and of arachic (*27*) acids but seldom for other acids.

The lead-salt-ether method, or Gusserow (7)-Varrentrapp (*29*) process, for the separation of liquid from solid acids is the most prominent illustration of the salt-soluble methods which, as a rule, have not proved sufficiently discriminative for quantitative use.

Fractionation of the acids *in vacuo* has failed as an analytical process.

Fractionation of ethyl esters appeared applicable to many fatty acids, although chemists generally consider the process as having little quantitative significance. The experience gained in purifying fatty acids indicated that the method was practicable and at least worthy of additional study.

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[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Reference is made by number (italic) to "Literature cited," pp. 731-732.

EARLIER INVESTIGATIONS

At the present writing esterification methods have so wide an application and contributors to the literature on the subject are so numerous that only references bearing directly on the analysis of oils and fats will be cited.

Rochleder (*28*) studied the action of absolute alcohol and dry hydrochloric acid gas upon castor oil in the separation of glycerol.

Berthelot (*2, p. 311–312*) applied the reaction to a number of glycerides and showed that esters of the fatty acids were formed in addition to glycerol.

Juillard (*15, p. 239*) prepared methyl and ethyl esters of dihydroxystearic acid by boiling the acid with 10 times its weight of alcohol and 2 or 3 drops of sulphuric acid.

Fischer and Speier (*5*) esterified various organic acids with different amounts of absolute ethyl alcohol and dry hydrochloric or concentrated sulphuric acid, also of methyl alcohol and hydrochloric acid and noted the yield. They did not deduce a general method.

Haller (*8–11*) heated a variety of oils and fats with twice their weight of absolute methyl alcohol containing 1 to 2 per cent of hydrochloric acid, removed the glycerol and excess alcohol with water, or preferably brine, and fractionated the esters up to 194° C. (methyl caprylate) at atmospheric pressure and the residual esters *in vacuo*. The methyl oleate present in the myristic and higher fractions was removed by chilling and absorption on porous plates. When complete alcoholysis was not obtained, he recommended a second treatment with a new portion of acidulated alcohol, the employment of a larger amount of alcohol at the outset, or the addition of an inert solvent such as ether to facilitate the reaction, particularly in the case of butter fat and of drying oils which readily oxidize and polymerize. The production of a small amount of aldehyde was noted in some instances. Haller neutralized the esters with barium carbonate or a solution of sodium carbonate and dried over calcium chloride or anhydrous sodium sulphate.

Phelps and Hubbard (*18*) esterified succinic acid with ethyl alcohol and hydrochloric acid, and secured the greatest yield with maximum dehydration. In other experiments (*19*) the addition of anhydrous zinc chlorid enhanced the reaction.

Complete esterification of 50 gm. of benzoic acid (*26*) was obtained by treating for 4 hours with 400 c. c. of absolute alcohol containing 1.25 per cent of dry hydrochloric acid and 10 gm. of anhydrous zinc chlorid, or for 3 hours with 200 c. c. of absolute alcohol and 2 gm. of sulphuric acid.

Several other chlorids (*24, p. 296–297; 20–25*) proved nearly as efficient as zinc chlorid under like conditions of operation.

Meyer (*17*) employed substantially the Haller method with cottonseed oil, but increased the amount of methyl alcohol to four times the weight of the oil, and obtained a yield of about 90 per cent.

Elsdon (*3, 4*) employed the Haller method with coconut oil and palm-kernel oil, fractionated the resulting methyl esters *in vacuo*, and refractionated to constant boiling point. He stated that the process had qualitative and a considerable amount of quantitative value, but was too lengthy for ordinary use.

Kailan (*16*) found that ether, benzene, and carbon tetrachlorid did not accelerate the esterification of benzoic acid with absolute alcohol and hydrochloric acid or with dilute alcohol and acid.

Wolff and Scholze (*30*) used a dilute sodium-bicarbonate solution to purify the esters when shaking out with ether.

Abderhalden and Kautzsch (*1*) esterified the silver salt of an amino acid by boiling with an excess of ethyl iodide.

Grandmougin, Havas, and Guyot (*6*) showed how an organic acid after treatment with sodium methylate might be converted by means of an excess of dimethyl sulphate into the methyl ester of the organic acid and sodium methyl sulphate.

Possibly methyl sulfonic acid might be substituted in some instances for dimethyl sulphate or methyl halide as indicated by an English patent, No. 9359.[1]

Hauser and Klotz (*12*) esterified organic acids with alcohols by passing the vapors over glucinum oxide heated to 310° C.

Permissible space does not allow one to do justice to the articles cited.

PRELIMINARY WORK

Further study of esterification was undertaken with a view of securing a method for determining the percentage of another insoluble acid besides stearic and oleic in butter fat. Lauric acid was naturally the most promising, on account of the lower boiling point of its esters, although myristic acid was also considered a possibility. The percentage of lauric acid, together with ordinary analytical data and the amount of stearic acid determined by crystallization, would permit a satisfactory calculation of the remaining insoluble acids.

MATERIAL.—At the outset the insoluble acids were employed for esterification with an idea that the previous elimination of soluble acids and of glycerol would be an advantage. In reality such did not prove the case, as water, a limiting factor in esterification, was produced in the reaction between fatty acids and alcohols, while glycerol was produced in the case of fats (glyceryl esters) and alcohols as shown by the following equations:

$$\underset{\text{fatty acid}}{RCOOH} + \underset{\text{alcohol}}{R_1OH} + \text{catalyzer} = \underset{\text{ester}}{RCOOR_1} + \underset{\text{water}}{H_2O}$$

$$\underset{\text{fat}}{(RCOO)_3C_3H_5} + 3\underset{\text{alcohol}}{R_1OH} + \text{catalyzer} = 3\underset{\text{ester}}{RCOOR_1} + \underset{\text{glycerol}}{C_3H_5(OH)_3}$$

[1] Chem. Abs., v. 6, no. 4, p. 535. 1912.

Furthermore, the employment of the original product was preferable from an analytical standpoint to say nothing of the time and labor

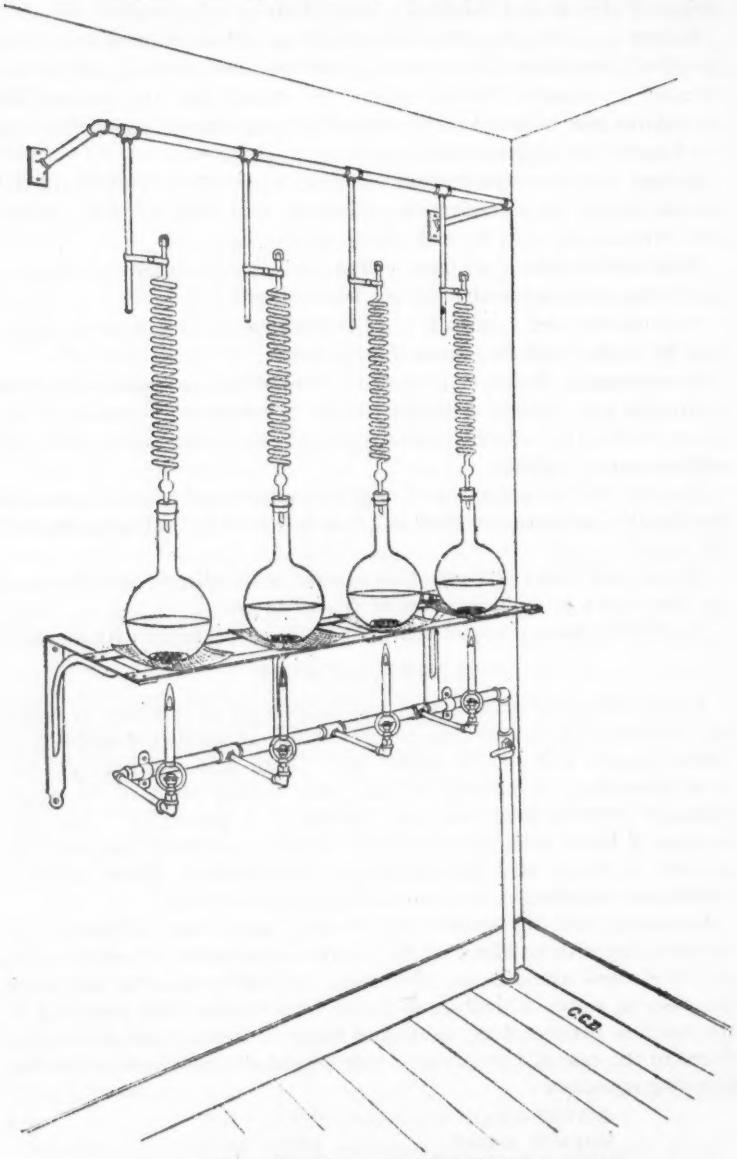

FIG. 1.—Apparatus employed in esterification.

involved in the preparation of a large stock of insoluble acids. Butter fat was used in all subsequent work.

ESTERIFICATION (fig. 1).—Earlier investigators have indicated various methods of esterification. Absolute alcohol with a mineral acid catalyzer appeared the most practicable for analytical purposes and was adopted tentatively. Between methyl and ethyl alcohols there was little choice, except as to cost and convenience, although the methyl esters have a somewhat lower boiling point. Ethyl alcohol was used exclusively.

The preparation of dry ethyl alcohol substantially free from aldehydes was found considerable of a problem. For dehydration neither metallic calcium nor any quicklime from the usual sources proved efficient. A granulated caustic lime containing about 95 per cent of calcium oxid was eventually obtained from the manufacturers which would produce a dry alcohol on the third distillation.

Different schemes have been suggested for the removal of aldehydes, such as oxidation with silver nitrate, potassium permanganate, or potassium bichromate, polymerization with caustic alkali or fractional distillation, and all were tried in some form or other. The following process finally proved satisfactory and was adopted:

Approximately 2 liters of alcohol were fractionated in a water bath over 600 to 700 gm. of caustic lime and 2 to 3 gm. of caustic soda. The main portion of the distillate, the first and last being rejected, was refractionated twice in a similar manner over fresh lime and soda. The rejected portions were united and retreated.

Dry hydrochloric acid or concentrated sulphuric acid has been the catalyzer almost invariably employed by different workers for esterification. The former with a greater hydrogen-ion concentration is apparently less efficient, gram for gram, than the latter, and is generally used in larger amounts. This may be due in part to loss of hydrochloric acid as ethyl chlorid by volatilization, but more likely to the dehydrating effect of sulphuric acid. By using a larger quantity of alcohol, together with certain chlorids, the yield of esters with hydrochloric acid may be increased, as shown by Phelps.

A considerable excess of alcohol is also required to insure the necessary mass action irrespective of its dehydrating action. For esterifying 150 gm. of butter fat, 400 c. c. of absolute ethyl alcohol containing 8 gm. of dry hydrochloric acid or 4 c. c. of concentrated sulphuric acid were employed. This amount of alcohol furnished about 11 times that needed for combination with the fatty acids.

The use of a neutral solvent, such as ether, did not appear to accelerate esterification and was omitted after a few trials. Butter fat diffused rapidly through the acid alcohol on boiling, and the solution generally cleared in a few minutes. Short boiling periods were tried, but 24 hours were considered more reliable and adopted.

PURIFICATION OF ESTERS.—After completing the esterification and cooling the solution, the esters must be precipitated and freed from mineral acid, glycerol, and excess alcohol.

Attention has already been called to various methods of neutralizing, purifying, and drying the esters, as described by other workers. Most of these processes and innumerable modifications were given careful trial, but the readiness with which at least a portion of the butter-fat esters hydrolyzed precluded the use of water in their purification. This was an extremely exacting condition and called for a salt soluble in alcohol, neutral in reaction, and with dehydrating properties. Dry magnesium

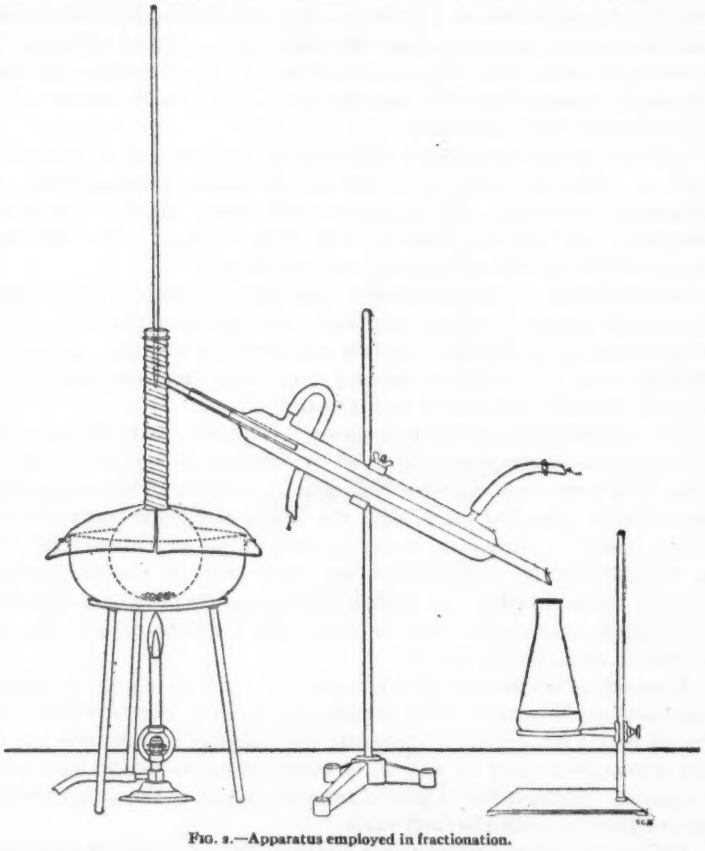

FIG. 2.—Apparatus employed in fractionation.

chlorid satisfactorily met these requirements, and furthermore was cheap and easily procured. On the addition of the dry salt in the presence of ether, a rapid separation of esters was obtained. The underlying solution was drawn off by means of a separatory funnel, and the esters were purified by "shaking out" several times with ether and a saturated alcoholic solution of magnesium chlorid. The ether facilitates the separation and should always be added before the magnesium chlorid.

All the agitation necessary can be obtained by reversing the separatory funnel several times, which gives a clear separation much quicker than a more violent shaking. The resulting esters contain ether and probably some alcohol, but do not require neutralizing or drying.

FRACTIONATION (fig. 2).—Fractionation of the esters *in vacuo* by means of a Bruehl or other type apparatus was found impracticable as a quantitative process for the reason that a constant level of the liquid in the distilling flask and a constant pressure were prime requisites for a definite fraction, neither of which could be successfully maintained with the facilities at hand. Furthermore, the use of such apparatus necessitates a certain aptitude or technic not possessed by all analysts.

Fractionation at atmospheric pressure required a high temperature, but proved feasible. Gas could not be used as a direct source of heat owing to fluctuations in pressure, influence of air currents, tendency to decompose the esters, etc., but when applied to a bath of superheated valve oil proved entirely satisfactory. This oil is a cheap commercial product that will safely withstand a temperature of over 400° C. when covered and may be used three times, possibly more, without appreciable loss of efficiency. All the exposed surface of the side-neck flask should be wound with asbestos paper to prevent chilling the vapors and breakage of the flask.

For some reason fractionation of the purified esters proved impracticable, possibly due to the influence of a relatively large amount of the higher esters particularly oleic, but was readily accomplished after a preliminary distillation. Accurate results are dependent in large measure on a slow, steady rise in temperature during the first distillation and subsequent fractionation. Glass beads were found helpful in boiling.

TABLE I.—*Boiling point and range of fractions (uncorrected) of esters of butter fat*

Ester.	Boiling point.	No.	Esters.	Range of fractions.
	°C.			°C.
Ethyl butyrate	119. 9–121	1	Ethyl butyrate, caproate, and oleate	125–180
Ethyl caproate	165 –167			
Ethyl caprylate	205 –208	2	Ethyl caproate, caprylate, and oleate	180–225
Ethyl caprate	243 –245			
Ethyl laurate	269	3	Ethyl caprylate, caprate, and oleate	225–270
Ethyl myristate	295			
Ethyl palmitate	?	4	Ethyl caprate, laurate, and oleate	270–300
Ethyl stearate	?			
Ethyl oleate	?	5	Ethyl laurate, myristate, and oleate	300–325
		6	Ethyl myristate, palmitate, and oleate	325–365

The range of the several fractions is more or less arbitrary, being dependent on the speed of distillation and the distance the vapors have to rise. The object was to secure fractions that did not contain more than two esters in addition to oleic ester, and, furthermore, adjacent fractions should contain approximately one-half of the saturated ester appearing

in each. The reported boiling point of the ethyl ester of a number of fatty acids that occur in butter fat, together with the range of the several fractions as determined by analysis for a "high" side-tube 500-c. c. distillation flask, may be noted in Table I.

The apparent lack of agreement is probably due to the greater distance the vapors have to rise in practical fractionation and to the influence of a gradually increasing amount of ethyl oleate in the several fractions. Analysis seemed to be the only method for accurately establishing the required range. Hempel tubes or similar apparatus could not be employed to break up the distillate.

As the preliminary work advanced, the results became more concordant and indicated that it was possible to determine not only lauric and myristic acids but also caproic, caprylic, and capric acids. A part of the butyric acid was recovered, but the main portion was evidently lost during the purification of the esters or distilled over with the ether, owing to the greater solubility and volatility of this ester.

ANALYSIS OF FRACTIONS.—Only the determinations of saponification and of iodin numbers by the usual methods employed with oils and fats were required.

CALCULATION OF RESULTS.—Having determined the weight, saponification and iodin numbers of the several fractions, the analyst must ascertain whether the range of the fractions had been accurately established. If correctly fractionated, the percentage of the different ethyl esters in the several fractions can be calculated algebraically and their weight computed, from which the percentage of the corresponding acids in the butter fat can be determined.

As a matter of convenience, the data necessary for these calculations have been compiled in Table II.

TABLE II.—*Fatty acids and their ethyl esters*

[C, 12.005; H, 1.008; O, 16.000; K, 39.10; I, 126.92]

Acid.	Molecular weight of acid.	Molecular weight of ester.	Saponification No. of ester.	Iodin No. of ester.	Reciprocal.	Conversion factor, ester to acid.
			Mgm.			
Acetic	60.042	88.084	636.983			0.68164
Butyric	88.084	116.126	483.165			.75852
Valeric	102.105	130.147	431.113			.78454
Caproic	116.126	144.168	389.185			.80549
Caprylic	144.168	172.210	325.812			.83716
Capric	172.210	200.252	280.187			.85997
Lauric	200.252	228.294	245.771			.87717
Myristic	228.294	256.336	218.885			.89060
Palmitic	256.336	284.378	197.301			.90139
Stearic	284.378	312.420	179.592			.91024
Arachic	312.420	340.462	164.800			.91764
Oleic	282.362	310.404	180.758	0.81777	1.22284	.90966
Erucic	338.446	366.488	153.096	.69263	1.44377	.92348
Linolic	280.346	308.388	181.940	1.64624	.60744	.90907
Linolenic	278.330	306.372	183.137	2.48561	.40232	.90847
Clupanodonic	276.314	304.356	184.350	3.33609	.29975	.90786
Ricinoleic	298.362	326.404	171.897	.77769	1.28586	.91409
Dihydroxystearic	316.378	344.420	162.906			.91858

As an illustration of the method of calculation, take the fifth fraction of a sample of butter fat (Table III) which weighed 50.69 gm., had a saponification number of 219.280 and an iodin number of 14.055, and was intended to contain ethyl laurate, myristate and oleate.

The iodin number, convertible to ethyl oleate by the factor 1.22284, is equivalent to 17.187 per cent.

Then 1.00 − 0.17187, or 0.82813, is the percentage of the residual esters in the fraction and 219.280 − (0.17187 × 180.758), or 188.213, their alkali-consuming power. The latter divided by the former gives a saponification number of 227, a figure between that of ethyl laurate (245.771) and that of ethyl myristate (218.885), as anticipated.

Let x indicate ethyl laurate and y ethyl myristate, then

$$x+y=0.82813$$
$$245.771x+218.885y=188.213$$
$$x=25.842 \text{ per cent of ethyl laurate}$$
$$y=56.971 \text{ per cent of ethyl myristate.}$$

The percentages 0.25842, 0.56971, and 0.17187 multiplied by the weight of the fraction (50.69 gm.), gives the weight of the several esters in the fraction 13.099, 28.879, and 8.712, which by their respective factors 0.87717, 0.89060, and 0.90966 may be converted into the weight of the several fatty acids in the fraction. The total weight of a fatty acid divided by the weight of fat taken (300) gives the percentage of that acid in the butter fat. The same method of calculation is followed in each of the other fractions.

METHOD IN DETAIL

REAGENTS.—Alcohol: absolute.

Hydrochloric acid: dry, generated by dropping concentrated sulphuric acid into a mixture of concentrated hydrochloric acid and sodium chlorid and dried by passing through concentrated sulphuric acid.

Sulphuric acid: concentrated, heated to 225° C.

Ethyl ether: anhydrous, freshly distilled over metalic sodium.

Magnesium chlorid: dry powder, neutral. Dried at 75° to 80° C.

Magnesium chlorid solution: 250 gm. of dry powder to 500 c. c. of absolute alcohol.

ESTERIFICATION AND PURIFICATION OF ESTERS.—Into a 1,000-c. c. flat-bottom globe flask are brought 150 gm. of filtered fat, together with 400 c. c. of alcohol previously charged with 8 gm. of dry hydrochloric acid, or 4 c. c. of concentrated sulphuric acid and a number (25) of glass beads. The flask is connected with a long spiral or other form of reflux condenser, and the mixture carefully boiled on 30-mesh Nichrome wire gauze for 24 hours.

After esterification the contents of the flask are cooled, 50 c. c. of ether and 150 gm. of magnesium chloride added, rotated to hasten saturation, transferred to a 1,000-c. c. pear-shaped separatory funnel and allowed to stand until a clear separation is secured. The underlying layer is drawn off into the original flask and the esters carefully shaken out two or three times with 25 to 50 c. c. of ether and 50 c. c. of an alcoholic solution of magnesium chlorid. Violent shaking causes a slow separation. After the removal of the final washing, the clear, purified esters are filtered through a firm, close-textured paper into a 500-c. c. round-bottom "low" side-tube distillation flask. The filter is extracted with ether which is run into the original globe flask containing the alcoholic layer and washings from the esters. More ether is added until a ready separation is obtained and the solution again transferred to the separatory funnel and allowed to stand several hours to recover any occluded esters which are then washed several times with ether and magnesium chlorid solution as previously described, and filtered into the distillation flask containing the first portion.

FRACTIONATION.—A number (50) of glass beads are placed in the side-neck distillation flask which is connected with a 12-inch Liebig condenser and heated in a bath of superheated valve oil for distillation of the esters. The exposed portion of the flask should be covered with asbestos paper and the condenser filled with cold water at the outset but no circulation should be permitted during the distillation. The temperature should be raised slowly. After the ether and alcohol are expelled, the entire distillate between 85° and 265° C. is collected and constitutes from 110 to 120 gm. with butter fat.

The distillate from two portions representing 300 gm. of butter fat and a number (50) of glass beads are brought into a 500-c. c. round-bottom "high" side-tube distillation flask which is connected with a Liebig condenser and heated as previously described. Particular care should be exercised in heating the oil bath so as to insure a slow, steady rise in temperature taking at least 80 minutes from the beginning of the first fraction to the completion of the last. The required range of every fraction must be accurately established with the apparatus employed by analysis (See Table I).

The volatility of the esters and the readiness with which they hydrolyze necessitates careful treatment of the fractions, which should be collected in tared flasks, weighed, and the saponification and iodin numbers determined as soon as possible.

APPLICATION OF THE METHOD

A sample of dry filtered butter fat churned from sweet cream from mixed milk of the Experiment Station herd was taken for examination. An analysis of the fat and of its insoluble acids gave the following results, which indicate a normal product.

FAT

Saponification number	mgm..	231.453
Acid number	mgm..	2.183
Ether number (e)	mgm..	229.270
Iodin number		27.999
Equivalent in oleic acid	per cent..	31.145
Total fatty acids (1.00−0.00022594 e)	per cent..	94.819
Insoluble fatty acids (by alcoholic saponification)	per cent..	87.500
Soluble fatty acids (by difference)	per cent..	7.319
Glycerol (0.00054703 e)	per cent..	12.542

INSOLUBLE ACIDS

Neutralization number	mgm..	221.890
Iodin number		28.125
Equivalent in oleic acid	per cent..	31.285
Stearic acid (by crystallization)	per cent..	13.010

The fat was esterified, with dry hydrochloric acid as a catalyzer, the esters purified and fractionated as described in the method.

In Table III are given the range, weight, saponification, and iodin numbers of the several fractions. From these data the percentage and weight of the different esters in the fractions were calculated (Table IV) and the weight of the corresponding acids and their percentage computed on the basis of the original fat (Table V).

TABLE III.—*Weight and analysis of fractions*

No.	Range of fraction.	Weight of fraction.	Saponification No.	Iodin No.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	125–180	5.0835	417.209	4.753	5.812
2	180–225	4.4400	346.396	8.091	9.894
3	225–270	6.8855	279.111	11.471	14.027
4	270–300	15.2990	240.983	12.846	15.709
5	300–325	50.6900	219.280	14.055	17.187
6	325–365	84.8800	205.955	16.691	20.410

TABLE IV.—*Percentage and weight of esters recovered*

No.	Ethyl butyrate.		Ethyl caproate.		Ethyl caprylate.		Ethyl caprate.		Ethyl laurate.		Ethyl myristate.		Ethyl palmitate.		Ethyl oleate.	
	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.
1	42.708	2.171	51.480	2.617											5.812	0.295
2			55.128	2.448	34.978	1.553									9.894	.439
3					38.210	1.942	57.763	3.977							14.027	.966
4							15.763	2.412	68.528	10.484					15.709	2.403
5									25.842	13.099	56.971	28.879			17.187	8.712
6											55.736	47.309	23.854	20.247	20.410	17.324
Total		2.171		5.065		3.495		6.389		23.583		76.188		20.247		30.139

TABLE V.—*Weight and percentage of fatty acids recovered*

Acid.	Gm.	Per cent.	Acid.	Gm.	Per cent.
Butyric acid (partial recovery)	1. 647	0. 549	Myristic acid	67. 853	22. 618
Caproic acid	4. 080	1. 360	Palmitic acid (partial recovery)	18. 250	6. 083
Caprylic acid	2. 926	. 975	Oleic acid (partial recovery)	27. 416	9. 139
Capric acid	5. 494	1. 831			
Lauric acid	20. 686	6. 895			

The recovery of ethyl butyrate was incomplete, owing to its high solubility and volatility; that of ethyl palmitate was due to inability to continue the distillation, owing to insufficient volume of higher esters and excessive heat requirements. Ethyl oleate appeared in all fractions, gradually increasing with the temperature.

The percentage of the different fatty acids in the butter fat are presented in Table VI. Butyric acid of the soluble acids and palmitic acid of the insoluble were determined by difference, stearic acid by crystallization, as previously stated, and oleic acid from the iodin number of the insoluble acids. No allowance was made for unsaponifiable matter in the calculation.

The alkali-consuming and glycerol-combining powers of the several acids are also recorded and confirm the results secured in large measure. The glycerol requirements of the constituent acids slightly exceed that of the fat, for the reason that no allowance was made for the free fatty acids present.

TABLE VI.—*Percentage of fatty acids in butter fat*

Fatty acids.	Amount present.	Saponification number.	Glycerol.
SOLUBLE ACIDS:	*Per cent.*	*Mgm.*	*Per cent.*
Butyric acid	[a] 3. 153	20. 084	1. 099
Caproic acid	1. 360	6. 571	. 359
Caprylic acid	. 975	3. 795	. 208
Capric acid	1. 831	5. 966	. 326
Total	.....	36. 416	1. 992
Calculated	7. 319	37. 299	.....
INSOLUBLE ACIDS:			
Lauric acid	6. 895	19. 319	1. 057
Myristic acid	22. 618	55. 588	3. 041
Palmitic acid	[a] 19. 229	42. 089	2. 302
Stearic acid	11. 384	22. 461	1. 229
Oleic acid	27. 374	54. 395	2. 976
Total	.....	193. 852	10. 605
Determined	87. 500	194. 154	.....
SOLUBLE AND INSOLUBLE ACIDS:			
Total	.....	230. 268	12. 597
Determined or calculated from fat	94. 819	231. 453	12. 542

[a] By difference.

In a later article attention will be called to additional analyses of butter fat and to the results reported by other workers employing different methods.

SUMMARY

The direct esterification of butter fat with subsequent fractionation of the resulting esters has proved an accurate and practical method for the determination of five of the fatty acids in butter.

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